

Amendments to the Specification

Please replace page 40 of the specification as follows:

		Cals Lines (MOL)																		
Priority Structure		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
M.	69	13515	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	
13	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	
24	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	13550	

Antimicrobial activity: On solid medium

Bacillus subtilis. 10 μ g/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

HRFABMS m/z 509.275351 [M-H₂O+H]⁺ (calcd for C₂₈H₃₇N₄O₅ 509.276396

Δ 1.0 mmu); LRFABMS using m-NBA as matrix m/z (rel intensity) 509 [M-H₂O+H]⁺ (5), 460 (2.7), 391 (3).

¹H NMR (CD₃OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, J = 2.0 Hz, H-21), 4.62 (dd, J = 8.4, 3.7 Hz, H-1), 3.98 (bd, J = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, J = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, J = 7.4 Hz, H-26);

¹³C NMR (CD₃OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Cells Lines (MöL/L)																				
Primary Screening		Prostate		Ovary		Breast		Melanoma		NSCL		Leukemia		Pancreas		Colon			Cervix	
		DU-145	LN-cuP	IGROV-1	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL					
Safracin P-22B	GI50	4.58E-06	3.08E-07	8.49E-07	3.02E-06	8.24E-07	5.20E-07	4.71E-06	1.13E-07	4.77E-06	1.01E-06	2.54E-06	6.95E-06	7.61E-07	4.65E-07					
	TGI	8.62E-06	6.08E-07	2.30E-06	7.04E-06	2.28E-06	9.99E-07	8.83E-06	4.67E-07	1.17E-05	2.75E-06	6.84E-06	1.90E-05	1.83E-06	9.32E-07					
	LC50	1.62E-05	1.20E-06	1.21E-05	1.65E-05	8.85E-06	2.01E-06	1.66E-05	1.84E-06	>1.90E-05	1.86E-05	1.84E-05	>1.90E-05	7.42E-06	1.86E-06					

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

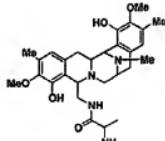
HRFABMS *m/z* 509.275351 [M-H₂O+H]⁺ (calcd for C₂₈H₃₇N₄O₅ 509.276396 Δ 1.0 mmu); LRFABMS using m-NBA as matrix *m/z* (rel intensity) 509 [M-H₂O+H]⁺ (5), 460 (2.7), 391 (3).

¹H NMR (CD₃OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, *J* = 2.0 Hz, H-21), 4.62 (dd, *J* = 8.4, 3.7 Hz, H-1), 3.98 (bd, *J* = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, *J* = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, *J* = 7.4 Hz, H-26);

¹³C NMR (CD₃OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Please replace page 41 of the specification as follows:

COMPOUND P-22A



Strain:

The same as for Br-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

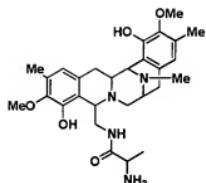
Biological activities of safracin P-22A

Antitumor activities

Antimicrobial activity: On solid medium

Bacillus subtilis, 10 μ g/disk (6mm diameter): NO ACTIVE

COMPOUND P-22A

*Strain:*

The same as for P-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

Biological activities of safracin P-22A

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemias	Pancreas	Colon			Cervix	
		DU-145	LNCaP	IGROV-1	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVODOX	HELA	HELA-APL
Safracin P-22A	GI50	>1.96E-05	4.19E-06	7.74E-06	1.30E-05	1.27E-05	5.93E-06	>1.96E-05	3.15E-06	>1.96E-05	1.26E-05	>1.96E-05	>1.96E-05	8.75E-06	7.66E-06
	TGI	>1.96E-05	9.26E-06	1.96E-05	>1.96E-05	>1.96E-05	1.33E-05	>1.96E-05	7.93E-06	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.96E-05
	LC50	>1.96E-05	1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05						

Antimicrobial activity: On solid medium*Bacillus subtilis.* 10µg/disk (6mm diameter): NO ACTIVE

Please replace page 43 of the specification as follows:

Biological activities of safracin P-19B

Antitumor activities

Primary Screening	Cells Lines (Möller)											
	3T3	4T1	143B	AT401	AT402	AT403	AT404	AT405	AT406	AT407	AT408	AT409
skBr3	600 ID	10500 10500										
MDA-MB-231	100 ID	10500 10500										

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): NO ACTIVE

Spectroscopic data:

HRFABMS *m/z* 495.260410 [M-H₂O+H]⁺ (calcd for C₂₇H₃₅N₄O₅ 495.260746 Δ 0.3 mmu); LRFABMS using m-NBA as matrix *m/z* (rel intensity) 495 [M-H₂O+H]⁺ (13), 460 (3), 391 (2); ¹H NMR (CD₃OD, 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, *J* = 7.1 Hz, H-26);

Biological activities of safracin P-19B**Antitumor activities**

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon		Cervix		
		DU-145	LN-cn ^P	IGROV-1	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-19B	GI50	1.70E-05	3.90E-06	5.42E-06	8.74E-06	7.08E-06	7.90E-06	>1.95E-05	2.38E-06	1.81E-05	1.55E-05	>1.95E-05	1.44E-05	6.73E-06	4.80E-06
	TG1	>1.95E-05	8.06E-06	1.48E-05	>1.95E-05	1.92E-05	>1.95E-05	>1.95E-05	5.77E-06	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.61E-05	1.00E-05
	LC50	>1.95E-05	1.67E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.40E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.95E-05

Antimicrobial activity: On solid medium*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE***Spectroscopic data:***

HRFABMS *m/z* 495.260410 [M-H₂O+H]⁺ (calcd for C₂₇H₃₅N₄O₅ 495.260746 Δ 0.3 mmu); LRFABMS using m-NBA as matrix *m/z* (rel intensity) 495 [M-H₂O+H]⁺ (13), 460 (3), 391 (2); ¹H NMR (CD₃OD, 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, *J* = 7.1 Hz, H-26);

Please replace page 46 of the specification as follows:

Activity Screening	Cells Lines (MöL)											
	1	2	3	4	5	6	7	8	9	10	11	12
PI - Penicille de la Cellule	GDS	0.25E+03	1.51E+03	2.00E+03	1.00E+03	1.75E+03	2.50E+03	4.00E+03	6.00E+03	4.75E+03	4.25E+03	3.25E+03
	TG	0.25E+03	4.51E+03	6.00E+03	3.00E+03	5.75E+03	8.50E+03	1.25E+04	1.85E+04	1.25E+04	1.25E+04	1.25E+04
	14051-C9	1.00E+03	1.51E+03	1.50E+03								

Activity Screening	Secondary Evaluation (MöL)											
	1	2	3	4	5	6	7	8	9	10	11	12
PI - Penicille de la Cellule	GDS	1.50E+03										

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS: *m/z* 509 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d, *J* = 7.2 Hz); ¹³C NMR (CDCl₃, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LNCaP	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANCI	HT29	LOVO	LOVODOX	HELA	HELA-APL	
Safracin D	GI50	5.22E-06	1.54E-06	2.68E-06	1.33E-06	4.71E-06	3.51E-06	6.04E-06	6.04E-07	4.77E-06	4.33E-06	6.99E-06	4.75E-06	3.76E-06	2.28E-06
	TGI	9.99E-06	4.12E-06	6.02E-06	3.34E-06	7.82E-06	6.21E-06	1.07E-05	1.16E-06	1.10E-05	1.79E-05	1.82E-05	8.85E-06	6.68E-06	5.24E-06
	LC50	1.90E-05	9.78E-06	1.35E-05	9.15E-06	1.30E-05	1.10E-05	1.88E-05	3.78E-06	>1.90E-05	>1.90E-05	>1.90E-05	1.65E-05	1.19E-05	1.21E-05

Secondary Evaluation (Mol/L)														
Secondary Screening				Macromolecules Synthesis				Apoptosis			DNA Binding			
				PROTEIN	DNA	RNA	NUCLEOSOMES	GEL						
Safracin D	IC50	1.90E-05	1.52E-05	3.80E-06	2.85E-06	6.65E-06								

Antimicrobial activity: On solid medium*Bacillus subtilis*. 10µg/disk (6mm diameter). Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS: m/z 509 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d, J = 7.2 Hz); ¹³C NMR (CDCl₃, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Please replace page 48 of the specification as follows:

Cells Lines (Molt.)														
Primer Concentration	S. Enteritidis		S. Typhimurium		E. Coli		Listeria		Pseudomonas		Candida		C. Albicans	
	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀
PM- Isentrichia Galacto														
100	1.050	1.050	1000	1.050	1000	1.050	1000	1.050	1000	1.050	1.050	1.050	1.050	1.050
100	>1.050	1.050	1000	>1.050	1000	>1.050	1000	>1.050	1000	>1.050	1.050	1.050	1.050	1.050
1000	>1.050	1.050	1000	>1.050	1000	>1.050	1000	>1.050	1000	>1.050	1.050	1.050	1.050	1.050

Secondary Evaluation (Molt.)														
Primer Concentration	S. Enteritidis		S. Typhimurium		E. Coli		Listeria		Pseudomonas		Candida		C. Albicans	
	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀	Dose	IC ₅₀
PM- Isentrichia Galacto														
100			1000		1000		1000		1000		1000		1000	

Antimicrobial activity: On solid medium

Bacillus subtilis. 10 μ g/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: m/z 511 [M+H]⁺; ¹H NMR ($CDCl_3$, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, $J = 6.6$ Hz); ¹³C NMR ($CDCl_3$, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN- caP	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL	
Safracin E	GI50	8.34E-06	3.86E-06	4.50E-06	4.54E-06	5.05E-06	3.94E-06	1.96E-05	4.25E-06	6.05E-06	7.89E-06	7.15E-06	5.07E-06	4.15E-06	4.03E-06
	TG1	1.96E-05	7.70E-06	8.85E-06	8.25E-06	9.24E-06	6.93E-06	>1.96E-05	8.21E-06	1.47E-05	1.96E-05	>1.96E-05	9.44E-06	7.29E-06	7.25E-06
	LC50	>1.96E-05	1.54E-05	1.74E-05	1.49E-05	1.70E-05	1.22E-05	>1.96E-05	1.59E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.75E-05	1.28E-05	1.30E-05

Secondary Evaluation (Mol/L)														
Secondary Screening				Macromolecules Synthesis				Apoptosis		DNA Binding				
				PROTEIN	DNA	RNA	NUCLEOSOMES	GEL						
Safracin E	IC50						1.57E-05	>1.96E-05						

Antimicrobial activity: On solid medium*Bacillus subtilis.* 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: *m/z* 511 [M+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, *J* = 6.6 Hz); ¹³C NMR (CDCl₃, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Please replace page 52 of the specification as follows:

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4; Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin B (OEt)

Antitumor activities

Mammalian Cells	Cells Lines (Möller)											
	HeLa	L929	3T3	MDA-MB-231	PC3	HT-1080	KB	HeLa	PC3	HT-1080	MDA-MB-231	3T3
Safracin B	65	1050	1050	1050	1050	1050	1050	1050	1050	1050	1050	1050
B	10	1050	>1050	1050	1050	1050	1050	1050	1050	1050	1050	1050
Dose	100	1050	1050	1050	1050	1050	1050	1050	1050	1050	1050	1050

Secondary Activity	Secondary Evaluation (Möller)											
	HeLa	L929	3T3	MDA-MB-231	PC3	HT-1080	KB	HeLa	PC3	HT-1080	MDA-MB-231	3T3
	1050	1050	>1050	1050	1050	1050	1050	1050	1050	1050	1050	1050

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

Spectroscopic data:

ESMS: m/z 551 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin B (OEt)

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening	Prostate			Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon				
	DU-145	LN- caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL	
Safracin B (OEt)	G150	4.01E-07	4.84E-08	4.06E-08	6.82E-07	4.82E-08	1.69E-07	5.01E-07	3.97E-08	6.49E-07	2.44E-07	4.43E-07	2.09E-06	8.92E-08	7.70E-08
		1.01E-06	>1.76E-05	9.97E-08	1.19E-06	1.16E-07	4.40E-07	1.16E-06	1.08E-07	2.06E-06	1.39E-06	1.09E-06	9.88E-06	3.15E-07	2.74E-07
	TGI	1.60E-05	8.28E-07	4.27E-06	6.37E-06	1.02E-06	1.13E-06	5.66E-06	3.69E-06	1.35E-05	>1.76E-05	>1.76E-05	>1.76E-05	1.35E-06	9.76E-07
Secondary Evaluation (Mol/L)															
Secondary Screening			Macromolecules Synthesis					Apoptosis			DNA Binding				
			PROTEIN		DNA	RNA		NUCLEOSOMES			GEL				
Safracin B (OEt)	IC50		>1.76E-05		1.76E-06	1.76E-07		5.28E-08			1.76E-05				

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

Spectroscopic data:

ESMS: m/z 551 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

Please replace page 54 of the specification as follows:

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

Antitumor activities

		Cells Lines [μM]																	
Primary Screening		HT1080	L929	PC3	DU145	LO2	HeLa	MDA-MB-231	SKBR3	SW420	HT1080	L929	PC3	DU145	LO2	HeLa	MDA-MB-231	SKBR3	SW420
Activity	Conc.	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50
Safracin A EtOAc (Eq.)	500	125.0	125.0	450.0	205.0	185.0	182.0	405.0	115.0	355.0	195.0	225.0	425.0	105.0	145.0	105.0	145.0	105.0	145.0
	100	25.0	25.0	90.0	35.0	35.0	35.0	75.0	8.5	75.0	45.0	55.0	95.0	25.0	35.0	25.0	35.0	25.0	35.0
	10	5.0	5.0	15.0	5.0	5.0	5.0	15.0	1.5	15.0	8.0	10.0	18.0	5.0	7.0	5.0	7.0	5.0	7.0
	1	1.0	1.0	3.0	1.0	1.0	1.0	3.0	0.3	3.0	1.5	2.0	3.5	1.0	1.5	1.0	1.5	1.0	1.5

		Secondary Evaluation [μM]																	
Secondary Screening		HT1080	L929	PC3	DU145	LO2	HeLa	MDA-MB-231	SKBR3	SW420	HT1080	L929	PC3	DU145	LO2	HeLa	MDA-MB-231	SKBR3	SW420
Activity	Conc.	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50	IC50
	500	50		50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
	100	10		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	10	2		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	1	0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Antimicrobial activity: On solid medium

Bacillus subtilis, 10μg/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon		Cervix		
		DU-145	LNCaP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANCI	HT29	LOVO	LOVODOX	HELA	HELA-APL
Safracin A (OEt)	G150	2.64E-06	3.78E-07	4.92E-07	2.01E-06	5.55E-07	7.96E-07	4.00E-06	3.11E-07	3.06E-06	1.97E-06	2.03E-06	5.72E-06	1.02E-06	7.64E-07
	TG1	5.39E-06	7.42E-07	9.28E-07	5.10E-06	1.16E-06	1.90E-06	7.17E-06	6.86E-07	5.83E-06	4.41E-06	4.41E-06	9.84E-06	2.91E-06	2.32E-06
	LC50	1.10E-05	1.45E-06	1.76E-06	1.30E-05	5.57E-06	5.77E-06	1.28E-05	1.51E-06	1.11E-05	9.88E-06	9.88E-06	1.69E-05	7.85E-06	6.69E-06

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin A (OEt)	IC50				6.33E-06	1.81E-06

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-EtOxy), 1.34 (t, J = 6.9 Hz, Me-EtOxy), 0.8 (d, J = 6.9 Hz, H-26)